Full title: Impact of a mild decrease in fasting plasma glucose on β-cell function in healthy subjects and patients with type 2 diabetes.

Abbreviated title: Mild decrease in plasma glucose and β-cell function

Marta Seghieri,1 Eleni Rebelos,1 Brenno D. Astiarraga,1 Simona Baldi,1 Andrea Mari,2 Ele Ferrannini.3

1 Department of Clinical & Experimental Medicine, University of Pisa, Italy
2 CNR Institute of Neuroscience, Padua, Italy
3 CNR Institute of Clinical Physiology, Pisa, Italy

Corresponding author: Marta Seghieri, Department of Clinical & Experimental Medicine, University of Pisa, Via Roma, 67, 56122, Pisa, Italy. Tel: 050/992151; fax: 050/553235; E-mail: martaseghieri@gmail.com

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Abstract

Restoring euglycaemia for weeks or months improves insulin secretion in patients with type 2 diabetes (T2D). We tested whether mild decrements in fasting glucose (FPG) acutely affect β-cell function and insulin sensitivity. Thirteen normotolerant (NGT) and ten T2D volunteered in paired: isoglycaemic test (Iso), after 100 min of stabilisation, an incremental glucose infusion over 3-hours was applied to raise plasma glucose to >22 mmol/L, followed by an arginine challenge; sub-isoglycaemic test (Sub), a glucose infusion matching the plasma glucose time-course of Iso was preceded by an insulin infusion period (100 min) aimed at maintaining a mild FPG reduction, while avoiding hypoglycaemia. β-cell function was assessed by mathematical modelling, whereas the acute insulin response (AIR) to arginine was determined from C-peptide levels. In Sub, FPG was lowered by 17% in NGT and 31% in T2D. On the glucose ramp, total insulin release was lower in Sub than Iso in both groups (from 106 [43] to 75 [39] nmol m⁻² in NGT and from 71 [63] to 64 [41] nmol m⁻² in T2D, p=0.001). In Sub, β-cell glucose sensitivity was significantly (p=0.008) reduced in NGT (from 50 [31] to 43 [21] pmol min⁻¹ m⁻² mM⁻¹) but not in T2D (19 [20] to 20 [20] pmol min⁻¹ m⁻² mM⁻¹). Likewise, AIR was lowered in NGT (8.9 [4.6] to 7.1 [4.4] nmol/L, p=0.048) but not in T2D (4.7 [3.3] to 5.3 [3.2] nmol/L). Insulin sensitivity improved in NGT but only marginally in T2D. Pre-stimulatory glucose levels acutely influence both β-cell function and insulin sensitivity, differentially in non-diabetic and type 2 diabetic individuals.

Key words: arginine-stimulated AIR, β-cell function, β-cell mass, fasting plasma glucose, insulin sensitivity.
Impaired β-cell function, a key factor in the progression from glucose intolerance to overt type 2 diabetes (T2D), could be the result of loss of β-cell mass (BCM), purely functional defects, or both.

Estimates of human BCM almost entirely rely on post-mortem measurements due to our present inability to assess BCM non-invasively via imaging or biomarkers. Autopsy specimens from T2D patients have shown decreases in the relative β-cell volume (4) or total mass (22) ranging from ~20 to 50%, while unchanged (15), moderately elevated (22) or markedly increased (25) have been observed in obese subjects compared with non-obese controls. Currently, β-cell function is variably assessed using indices derived from fasting plasma insulin and glucose concentrations, dynamic tests with oral glucose or standard meals, and responses to intravenous glucose infusions and/or non-glucose secretagogues. Ward et al. (31) evaluated β-cell function of T2D and normotolerant (NGT) subjects by measuring the acute insulin response to 5 g of intravenous arginine (AIR) at 5 matched glucose levels ranging from 5.5 to 30 mmol/l. By fitting AIR to glucose levels, an asymptote was achieved in NGT subjects at glucose levels higher than 20 mmol/l; this condition was suggested to elicit maximal insulin secretion, and was therefore taken as an estimate of BCM (31). However, in these studies insulin secretion might have been further stimulated – even in nondiabetic subjects – by the addition of other secretagogues, e.g., sulfonylureas or GLP-1.

Moreover, in these studies the impact of pre-stimulatory glucose levels on the stimulated β-cell responses was not taken into consideration. For example, in healthy women undergoing a glucose-dependent arginine stimulation and a euglycemic clamp, baseline and maximal arginine-induced insulin secretion rose at higher glucose levels, while insulin sensitivity was depressed (1). In T2D, it has been repeatedly shown that restoring normoglycemia with insulin can improve the insulin secretory responses to glucose and non-glucose stimuli. Thus, in an early study in T2D patients (29), 20 hours of glycemic normalisation were sufficient to induce an increased response to glucose, while that to tolbudamide was unaffected. In another study in poorly controlled T2D patients (10),
the mean 24-hour integrated insulin and C-peptide concentrations evaluated after 3 weeks of continuous subcutaneous insulin infusion were markedly increased. In yet another study in T2D patients with poor glycemic control, near-normalisation of blood glucose for 4 weeks greatly improved β-cell response to both GLP-1 and GIP infusion but not to glucose alone (14). These relatively rapid changes in insulin secretory responses are unlikely to be caused by changes in BCM.

On the converse side, the effects of hyperglycemia on glucose-stimulated insulin secretion are controversial. When hyperglycemic clamps (16) or graded glucose infusions (8) were performed in obese non-diabetic humans an increase above basal level in β-cell function was observed. However, when plasma glucose levels were clamped at euglycemic or progressively increasing hyperglycemic levels for 68 hours, an impairment of insulin secretory function and insulin sensitivity was observed at glucose concentration between ~ 9 and 12 mmol/l (3). This was taken to represent the emergence of glucose toxicity. On the other hand, in healthy volunteers subjected to experimental 72-96 hours of hyperglycemic hyperinsulinemia, the insulin secretory response to an acute glucose challenge was more than doubled (7).

Taken together, these studies suggest that the pre-stimulatory level of plasma glucose is one of the immediate, acute determinants of the secretory response of the β-cell to subsequent stimulation. The present study was therefore undertaken to test this hypothesis in both NGT and T2D individuals under strictly controlled experimental circumstances.

Methods

Subjects Thirteen subjects with normal glucose tolerance (NGT) and ten type 2 diabetic (T2D) patients were studied, with a one-week interval between the tests. All participants were male. Inclusion criteria were: age 18-65 years, body mass index (BMI) 20-35 kg/m², non-smoking, free from cardiovascular/pulmonary/hematological/renal/hepatic disease. The T2D patients had never been treated with insulin, and oral medications (metformin, sulfonylureas and sitagliptin) were
withdrawn 24-48 hours before each study. No changes in diet, weight or lifestyle were allowed from the time of recruitment until the end of the studies. All tests were performed after an overnight (10-12 hours) fast. The nature and purpose of the study were carefully explained to all participants before they provided written consent to participate. The study procedures were approved by the Institutional Ethics Committee of Pisa University.

Study protocol Subjects were admitted to the metabolic ward at 8:00 am on two occasions in this order: isoglycemic (Iso) and sub-isoglycemic (Sub) test. A catheter was inserted into an antecubital vein for the infusion of insulin, glucose, and arginine. A second catheter was inserted retrogradly into a vein of the dorsum of the hand for collection of blood samples; the hand was placed in a thermo-cover at ∼60 °C to achieve arterialization of venous blood.

Baseline infusion period (-100 to 0 min) In the Iso test, saline was infused throughout this period of time, while in the Sub test a variable infusion of regular insulin (Humulin R, Eli Lilly&Co., Indianapolis, USA) was applied to lower fasting plasma glucose concentrations (FPG) within the non-hypoglycemic range.

Glucose ramp (0 to 180 min) In the Iso test, starting at time 0 glucose (as a 20% D-glucose solution) was infused at a variable rate to create a quasi-linear increase in plasma glucose concentrations to peak values of ∼22 mmol/l (in NGT) and ∼28 mmol/l (in T2D) over 3 hours by using an ad hoc algorithm (19). In the Sub test, at time 0 the insulin infusion was stopped, and the exogenous glucose infusion was started and adjusted to match the plasma glucose profile of the Iso test as closely as possible. Blood samples were drawn every 10 min for glucose measurement and every 20 min for plasma insulin and C-peptide determination.

Arginine challenge (180 to 190 min) In both tests, at time 180 min a 5-g arginine bolus (L-arginine HCl, diluted in sterile water) was injected, while glucose infusion was maintained at the same rate as that of time 180 min. Following the bolus, blood samples were collected at 1, 2, 3, 4, 5, 6, 8 and 10 min.

Analytical procedures Plasma glucose was measured by the glucose oxidase technique
(Beckman Glucose Analyzers; Beckman, Fullerton, CA, USA). Plasma insulin and C-peptide were measured by an electro-chemiluminescence assay on a COBAS e411 (Roche, Indianapolis, IN, USA). Serum potassium was measured by an ion-selective electrode. Blood samples for C-peptide and insulin were drawn using a protease inhibitor (5 μl/ml of 10 mg/ml gabexate mesylate).

Calculations. Insulin secretion rates (ISR) were reconstructed from plasma C-peptide concentrations using the two-exponential model proposed by Van Cauter et al. (30), in which the model parameters are individually adjusted to the subject's anthropometric data. β-cell glucose sensitivity was calculated as the mean slope of the dose-response of ISR vs plasma glucose concentrations during the 0-180-min time interval (17). The insulin response during the arginine challenge was estimated as the integral of absolute plasma C-peptide concentrations over the 10 min following arginine injection, calculated by trapezoidal rule; mean C-peptide concentrations over this time period were calculated as the ratio of the time integral to the time interval.

The exogenous glucose infusion rate (GIR) was expressed as the mean value normalized by FFM. The metabolic clearance rate of glucose (MCRG) was obtained as the ratio of GIR and mean plasma glucose concentration. Insulin sensitivity was estimated as the ratio between MCRG and the mean insulin concentration (log-transformed). GIR, MCRG and insulin sensitivity were calculated over the 40-180-min time interval, as discussed below.

Statistical analysis. Data are given as means±SEM (or median [interquartile range] for variables with a skewed distribution). Groups were compared by the Mann-Whitney test. Time series were analyzed by ANOVA for repeated measures; for these tests, variables with skewed distribution were log-transformed. Group differences over time series were analyzed by 2-way ANOVA for repeated measures. Statistical analyses were performed using JMP®7.0; a p value < 0.05 was considered statistically significant.

Results
The T2D patients were older than the NGT subjects. BMI ranged from 17 to 33 kg/m² for NGT subjects and from 25 to 34 kg/m² in the T2D group, while fat mass ranged from 3.6 to 29.9 kg and 12.5 to 35.0 kg for NGT and T2D, respectively. The clinical phenotype of T2D subjects included raised serum triglyceride and transaminase levels and lower HDL-cholesterol levels (Table 1).

**Basal infusion period (-100 to 0 min).** During the initial 100 min of the **Sub** test, insulin was infused at a mean rate of 0.42 ± 0.03 and 0.80 ± 0.10 mU kg⁻¹ min⁻¹ in NGT and T2D subjects, respectively, which lowered plasma glucose concentrations at time 0 by 17 ± 2% and 31 ± 3% (Table 2). In the **Sub** test, plasma insulin concentration was ~4-fold higher at time 0 than at time -100 in NGT participants, and 2-fold higher in T2D patients.

In the NGT group, the difference in serum potassium concentrations between basal and the end of the 100 min insulin infusion (time 0) was -0.44±0.17 mmol/L in the **Sub** test vs 0.10±0.08 mmol/L in the **Iso** test (p=0.0134), whereas in the T2D group the corresponding changes were -0.24±0.09 and -0.03±0.22 (p=0.273).

**Glucose ramp (0 to 180 min)** In the **Iso** study, plasma glucose levels rose to a peak value of 21.6 ± 0.6 mmol/l in NGT and 27.1 ± 0.6 mmol/l in T2D; the glucose ramps were closely reproduced in the **Sub** study in both groups of subjects (Fig. 1). Plasma insulin concentrations rose gradually during the ramp, but the mean insulin level was significantly lower in the **Sub** compared to the **Iso** test in both groups. In both groups in the **Sub** test, the GIR time-course described a shoulder over the initial 30-40 min of the ramp followed by a linear increase (Fig, 2). The initial excess GIR was presumably due to compensating for the suppression of endogenous glucose release (induced by the pre-infusion of exogenous insulin) as well as the need to jump to the **Iso** values from lower levels. By 40 min into the ramp, plasma insulin and glucose concentrations (126 ± 28 pmol/l and 9.3 ± 0.2 mmol/l, respectively, in NGT, and 168 ± 35 pmol/l and 12.0 ± 0.4 mmol/l in T2D) were high enough to fully suppress endogenous glucose release (6). The mean GIR over 40-180 min and its ratio to the mean plasma glucose levels (glucose clearance) were only marginally, but not statistically significantly increased during **Sub** compared to **Iso**. When accounting for the
concomitant plasma insulin concentrations – thereby indexing insulin sensitivity – T2D patients were more insulin resistant than NGT subjects; in the Sub test insulin sensitivity improved significantly vs the Iso test in both groups (Table 2).

Fasting insulin secretion (time -100 min) was higher in T2D than NGT (136 [72] vs 70 [35] pmol min\(^{-1}\) m\(^{-2}\), p<0.004). At the end of the insulin pre-infusion period, insulin secretion (at time 0) was reduced from fasting values by 60 ± 6% in NGT and 45 ± 4% in T2D (Table 2). Over the 3-hour glucose ramp, insulin secretion rose progressively in both groups, but total insulin release was significantly lower in the Sub than the Iso test (Fig. 3). When insulin secretion rates were analyzed in the context of the concomitant plasma glucose levels, β-cell glucose sensitivity was lower in T2D than NGT in the Iso study, but was significantly reduced (by 19 ± 3%) on the Sub test (Fig. 4) only in NGT.

**Arginine challenge** The C-peptide response to arginine was lower in T2D than NGT on the Iso test, but was reduced on the Sub test only in NGT subjects (Table 2).

**Discussion**

Our main finding is that, in nondiabetic subjects an acute, modest reduction in fasting plasma glucose concentration – still within the range of euglycemia – causes a decrease in insulin secretion in response to progressive hyperglycemia (and to superimposed arginine), a decrease in β-cell glucose sensitivity, and an enhancement of insulin sensitivity. Because of the isoglycemic protocol, this secretory modulation could not be attributed to differences in glucose exposure but solely to the short period of insulin pre-infusion. Because fasting glucose levels were lowered using insulin, our findings could be explained by the hyperinsulinemia or the relative hypoglycemia. Previous studies (11, 18) have shown that steady-state physiologic hyperinsulinemia applied under euglycemic clamp conditions potentiates insulin secretion, and that this effect is blunted in insulin resistant subjects. These studies, however, cannot be directly compared with our study, where only the pre-stimulatory period was exposed to mild hyperinsulinemia.
The cellular mechanisms underlying our findings cannot be directly identified from *in vivo* experiments. In a systematic series of experiments with human islets, Henquin et al. (13) found that first-phase insulin response to a step increase in glucose (to 15 mmol/L) was inversely related to the prestimulatory glucose concentration, confirming *in vivo* results in healthy volunteers (28). The key difference between these previous studies and the present work is that the gradual glucose ramp design does not elicit a detectable first phase – which is the canonical response to a step rise of glucose concentrations to some pre-set, arbitrary level – but rather induces a progressive stimulation of insulin release (Fig. 1). This mode of secretory response, which is rather akin to the second-phase insulin response to a hyperglycemic clamp, is not as strongly dependent on pre-stimulatory glucose concentrations *in vitro* (13). Furthermore, a glucose ramp is closer to free-living conditions, which never reproduce step functions of glycemia (9). In fact, using a sequential protocol Halter et al. (12) found a reduced insulin response to isoproterenol following a brief period of insulin-induced hypoglycemia. In addition, Pfeiffer et al. (21) reported that, when the steady-state pre-stimulus glucose concentration was lowered by a 0.33 mU·kg\(^{-1}\)·min\(^{-1}\) insulin infusion (similar to ours) or raised by a 900 mg/min glucose infusion, no effect on first-phase insulin secretion was observed. In contrast, the second phase response to intravenous glucose fell during insulin infusion and increased during the glucose infusion. Overall, a linear relationship was found between the change of pre-stimulus glucose level from the control to that during the insulin or glucose infusion and the change in second phase response. Our protocol, on the other hand, explored the effect of a brief exposure to mild sub-euglycemia on the secretory response at matched glucose levels over a range of glycemias from normal to near-maximal. Thus, it can be concluded that *in vivo* β-cells react to even short, mild sub-euglycemia by downregulating their glucose sensing over a subsequent period of at least 3 hours. Furthermore, this kind of carry-over or memory extends to the response to arginine.

This phenomenon would be compatible with the paradigm that antecedent glycemia controls the rate of exchange between immediately releasable and readily releasable pools of insulin granules as
well as the rate of hormone supply to these pools through the exocytotic machinery \((5,26,27)\); an intrinsical functional attribute of β-cell sensing, however, could also explain it \((23)\). On the other hand, changes in signaling (metabolism and cytosolic Ca) could be involved in the observed changes in insulin secretion \((13)\). Alternatively – or additionally – the insulin-induced decrement in serum potassium concentrations measured during the pre-stimulatory phase may have contributed to down-regulate the subsequent glucose-induced insulin response; this mechanism has \textit{in vivo} precedents \((24, 20)\).

In our T2D patients, β-cell glucose sensitivity was impaired (by \(~60\%\) on average) and the secretory response to arginine was 50\% lower compared to the NGT subjects (Table 2). In contrast to the finding in NGT participants, however, in T2D patients there was no clear effect of antecedent glycemia on either β-cell glucose sensitivity or the arginine response. This outcome is compatible with the notion that in diabetic β-cells priming or mobilization of the readily releasable pool of insulin granules may be abnormal and require prolonged normalization of glucose levels to be improved \((5)\). The finding is also compatible with the non-significant change in serum potassium levels observed in T2D patients during pre-stimulation, another sign of insulin resistance. In fact, \textit{in vivo} studies, lowering glycemia by insulin \((10)\) or bariatric surgery \((2)\) in T2D patients improves β-cell function over periods of weeks or months.

With regard to insulin sensitivity, rates of exogenous glucose infusion were similar in the Iso and Sub test. However, when these rates – representing whole-body tissue glucose disposal plus any glycosuria – were normalized by the concomitant plasma glucose and insulin concentrations (thereby indexing insulin sensitivity), T2D patients were insulin resistant compared to NGT subjects, as expected. Furthermore, on the Sub protocol insulin sensitivity was enhanced in the NGT group but were roughly maintained in the T2D group (Table 2). In previous studies, sustained (72 hours) hyperinsulinemia with maintenance of euglycemia was associated with a worsening of insulin sensitivity \((7)\). Thus, a possible explanation of our finding is that lowering endogenous plasma insulin \textit{via} sub-euglycemia may have resulted in improved insulin sensitivity
The current results negate the proposition that an arginine bolus given on top of marked hyperglycemia may constitute an *in vivo* quantitation of maximal insulin secretory capacity and, by inference, BCM. In fact, in our study subjects the brief antecedent plasma glucose lowering was sufficient to cut maximal insulin secretion by ~30%, which obviously cannot be due to changes in BCM. It must also be considered that the true ‘maximal’ insulin secretory capacity might only be approximated by adding multiple secretagogues (arginine, sulphonylureas and GLP-1) on top of prolonged, maximal hyperglycemia. Therefore, while this and similar protocols can still differentiate between subjects with NGT and diabetic patients – as originally shown by Ward *et al.* (31) – they fall short of providing even approximate estimates of BCM.

In summary, the current results provide evidence that β-cell responsivity to glucose and non-glucose stimuli is inherently dependent on the pre-stimulatory glucose exposure; such conditioning differs in strength and time-course between non-diabetic subjects and T2D patients.
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Disclosures The authors declare that there is no duality of interest associated with this manuscript.

Author Contributions MS, ER, BDA, SB and AM acquired and analysed the data. MS, ER, BDA drafted the article. EF reviewed and edited the article. EF and AM were responsible for the conception of the study. All authors approved the final version.
References


Fig. 1 – Plasma glucose and C-peptide concentrations during 100 min of isoglycemia (Iso, dotted lines) or sub-euglycemia (Sub, full lines) and during 180 min of glucose ramping in normotolerant subjects (NGT) and patients with type 2 diabetes (T2D). The insets show the corresponding responses to arginine administration.

Fig. 2 – Exogenous glucose infusion rates during 180 min of glucose ramping following isoglycemia (Iso, dotted lines) or sub-euglycemia (Sub, full lines) in normotolerant subjects (NGT) and patients with type 2 diabetes (T2D).

Fig. 3 – Insulin secretion rates in the pre-stimulatory phase and during 100 min of isoglycemia (Iso, dotted lines) or sub-euglycemia (Sub, full lines) and during 180 min of glucose ramping in normotolerant subjects (NGT) and patients with type 2 diabetes (T2D).

Fig. 4 – β-cell glucose sensitivity at isoglycemia (Iso, dotted lines) or sub-euglycemia Sub, full lines) in normotolerant subjects (NGT) and patients with type 2 diabetes (T2D).
Table 1 – Anthropometric and biochemical characteristics of subjects. *

<table>
<thead>
<tr>
<th></th>
<th>NGT</th>
<th>T2D</th>
<th>p°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27 ± 1</td>
<td>60 ± 3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>24.5 ± 1.2</td>
<td>29.4 ± 0.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>-</td>
<td>7 ± 2</td>
<td>-</td>
</tr>
<tr>
<td>Fat-free mass (%)</td>
<td>83 ± 2</td>
<td>72 ± 1</td>
<td>0.0005</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>5.2 ± 0.1</td>
<td>8.1 ± 0.8</td>
<td>0.0004</td>
</tr>
<tr>
<td>Fasting plasma insulin (pmol/l)</td>
<td>37 ± 5</td>
<td>127 ± 43</td>
<td>0.001</td>
</tr>
<tr>
<td>Fasting plasma C-peptide (nmol/l)</td>
<td>0.50 ± 0.05</td>
<td>1.30 ± 0.28</td>
<td>0.004</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>-</td>
<td>60.8 ± 2.3</td>
<td>-</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.1 ± 0.2</td>
<td>4.3 ± 0.3</td>
<td>ns</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.4 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.8 ± 0.1</td>
<td>1.8 ± 0.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Alanine aminotransferase (U/l)</td>
<td>17.3 ± 2.7</td>
<td>26.0 ± 4.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/l)</td>
<td>18.8 ± 1.2</td>
<td>20.6 ± 2.8</td>
<td>ns</td>
</tr>
<tr>
<td>γ-glutamyltransferase (U/l)</td>
<td>17.8 ± 2.5</td>
<td>37.5 ± 10.8</td>
<td>0.04</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.83 ± 0.02</td>
<td>0.72 ± 0.01</td>
<td>ns</td>
</tr>
</tbody>
</table>

* Data are mean±SEM; ° for the comparison of NGT vs T2D patients by Mann-Whitney test.
Table 2 – β-cell function and insulin sensitivity data.*

<table>
<thead>
<tr>
<th>Glucose ramp</th>
<th>NGT</th>
<th>T2D</th>
<th>p*</th>
<th>Group</th>
<th>Test</th>
<th>GxT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 0 glucose (mmol/l)</td>
<td>5.3 ± 0.1</td>
<td>4.3 ± 0.1</td>
<td>8.1 ± 0.8</td>
<td>5.7 ± 0.6</td>
<td>0.002</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time 0 ISR (pmol min⁻¹.m⁻²)</td>
<td>68 [38]</td>
<td>24 [15]</td>
<td>131 [90]</td>
<td>66 [36]</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean glucose (mmol/l)§</td>
<td>13.3 ± 0.1</td>
<td>13.5 ± 0.2</td>
<td>17.4 ± 0.3</td>
<td>17.4 ± 0.3</td>
<td>&lt;0.001</td>
<td>0.222</td>
</tr>
<tr>
<td>Mean insulin (pmol/l)</td>
<td>561 [537]</td>
<td>281 [507]</td>
<td>239 [386]</td>
<td>202 [255]</td>
<td>0.336</td>
<td>0.019</td>
</tr>
<tr>
<td>Total IS (nmol·m⁻²)</td>
<td>106 [43]</td>
<td>75 [39]</td>
<td>71 [63]</td>
<td>64 [41]</td>
<td>0.127</td>
<td>0.001</td>
</tr>
<tr>
<td>Max. ISR (pmol min⁻¹.m⁻²)</td>
<td>969 [493]</td>
<td>837 [450]</td>
<td>491 [441]</td>
<td>474 [403]</td>
<td>0.008</td>
<td>0.261</td>
</tr>
<tr>
<td>β-cell GS (pmol min⁻¹·mm⁻¹)</td>
<td>50 [31]</td>
<td>43 [21]</td>
<td>19 [21]</td>
<td>20 [20]</td>
<td>0.001</td>
<td>0.465</td>
</tr>
<tr>
<td>GIR (μmol·kg⁻¹·min⁻¹)§</td>
<td>125 [19]</td>
<td>132 [40]</td>
<td>79 [38]</td>
<td>90 [36]</td>
<td>&lt;0.001</td>
<td>0.134</td>
</tr>
<tr>
<td>MCR₆ (ml·kg⁻¹·min⁻¹)§</td>
<td>7.7 [0.9]</td>
<td>8.5 [2.3]</td>
<td>3.8 [2.2]</td>
<td>4.8 [2.0]</td>
<td>&lt;0.001</td>
<td>0.167</td>
</tr>
<tr>
<td>Insulin sensitivity (units)§</td>
<td>1.28 [0.22]</td>
<td>1.47 [0.34]</td>
<td>0.78 [0.35]</td>
<td>0.80 [0.36]</td>
<td>&lt;0.001</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Arginine challenge

| Mean C-peptide (nmol/l) | 8.9 [4.6] | 7.1 [4.4] | 4.7 [3.3] | 5.3 [3.2] | 0.030 | 0.041 | 0.048 |

* Data are mean±SEM or median [interquartile range]; ISR = insulin secretion rate; IS = insulin secretion; β-cell-GS = β-cell glucose sensitivity; GIR = glucose infusion rate; MCR₆ = metabolic clearance rate of glucose; Insulin sensitivity index (in units of ml·kg⁻¹·min⁻¹·ln[pM⁻¹]).

§ p value for NGT vs T2D (Group), Iso vs Sub (Test) and their interaction (GxT) by 2-way ANOVA for repeated measures of log-transformed variables.

§§ mean value between 40-180 min.
Figure 2

Glucose infusion rate (µmol·kg\(^{-1}·min\(^{-1}\))

Time (min)

NGT

T2D

Iso

Sub
Figure 3

Insulin secretion rate (pmol/min⁻¹·m⁻²)

NGT

- Iso
- Sub

T2D
Figure 4

Plasma glucose (mmol/l) vs. Insulin secretion rate (pmol·min⁻¹·m⁻²)

- NGT
- T2D

Legend:
- Iso
- Sub